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Studies on the microflora of Danish beech forest soils

IV. Yeasts and yeast-like fungi

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With 27 figures in the text

The present paper contains observations on the occurrence of yeasts and yeast-like fungi, which were made in connection with qualitative examinations of the bacterial and fungal flora of beech forest soils, supplemented with results of enrichment culture experiments. Furthermore descriptions are given of a number of yeast strains, isolated from the investigated localities. Descriptions of these localities are found in a previous paper (V. JENSEN 1963a).

The yeasts and yeast-like fungi do not constitute a well-defined group. The boundaries between this and neighbouring groups are often vague and arbitrary. In the present paper the concepts of LODDER and VAN RIJ (1952) and WINDISCH (1960) are used, and e. g. the so-called "black yeasts" are not included in the study.

Numbers of yeasts in the soils

A number of plate counts have been made on the following media: Thornton's mannitol asparagine salts agar (THORNTON 1922), soil extract agar (prepared from equal parts of garden soil and tap water, with addition of 0.02 per cent dipotassium phosphate, pH 6.7), tryptone glucose extract agar (Bacto), and dextrose peptone yeast extract agar (DPYA), a selective medium for counts of fungi (PAPAVIZAS and DAVEY 1959).

After counting, a number of transfers were made from isolated colonies, usually one hundred in each experiment, and the resulting cultures were examined and classified (for further details, see V. JENSEN 1963b). The percentages of yeast cultures were also recorded. This permits an estimation of the number of yeasts in the soils which are capable of growth on the respective plating media (see tables 1 and 2).

The results indicate that Thornton's agar is an unsuitable medium for yeasts, since much lower counts were obtained on this than on the other media. The highest numbers were found on soil extract agar and on tryptone glucose extract agar. Somewhat lower numbers were obtained on DPYA. In fact most yeasts do not grow very well on this medium, and the colonies formed are usually small.

The numbers of yeasts range from 0 to c. 100,000 per gm. of dry soil for mull

Table 1
Plate counts on beech mull soils

Plating medium	Locality	Date of sampling	Soil moisture % of d. m.	Soil pH	Plate counts mill. per gm. of dry soil	Yeasts	
						percentage	thousands per gm. of dry soil
Thornton's agar	I	13. 4. 56	45	5.1	9.5	0	0
	III	3. 5. 56	54	—	3.5	0	0
	VI	3. 5. 56	39	6,9	20.6	0	0
	VII	6. 4. 57	37	5,4	5.4	1.7	92
	X	6. 4. 57	56	4.7	0.8	0	0
Soil extract agar	I	13. 4. 56	45	5.1	7.2	1.0	72
	III	3. 5. 56	54	—	3.9	0	0
	VI	3. 5. 56	39	6.9	8.7	1.0	87
	VII	6. 4. 57	37	5.4	5.6	1.7	95
	X	6. 4. 57	56	4.7	1.2	1.5	18
	VI	5. 5. 58	52	5.3	16.6	0	0
	I	3. 7. 58	25	5.4	10.3	0	0
	III	2. 12. 58	43	4.7	2.5	1.0	25
Trypt. gluc. extract agar	I	13. 4. 56	45	5.1	5.1	1.0	51
	III	3. 5. 56	54	—	2.4	3.0	72
	VI	3. 5. 56	39	6.9	7.2	1.0	72
	VII	6. 4. 57	37	5.4	11.2	1.0	112
	X	6. 4. 57	56	4.7	2.0	2.0	40
DPYA	I	16. 3. 61	42	5.1	0.8	1.0	8
	—	2. 6. 61	41	5.0	0.6	1.0	6
	—	8. 8. 61	39	5.0	0.6	5.0	30
	—	27. 9. 61	36	4.8	0.8	1.0	8
	—	5. 12. 61	43	5.1	0.7	6.0	42
	—	1. 2. 62	46	5.0	1.4	5.0	70
	III	22. 3. 61	47	5.3	0.6	5.0	30
	—	29. 5. 61	35	4.9	0.6	0	0
	—	11. 8. 61	40	5.0	0.8	4.0	32
	—	28. 9. 61	23	4.9	0.7	3.8	27
	—	8. 12. 61	53	5.1	0.8	9.0	72
	—	2. 2. 62	52	4.7	0.4	14.0	56

localities, and from 0 to c. 500.000 for mor localities[1]). On an average the numbers were 3—4 times higher in mor than in mull soils.

Of course, the numbers found by this method must be regarded only as crude estimates. As in all plate counts, however, an underestimation is far more probable than an overestimation. Therefore, the results can be considered as minimum numbers with a fair degree of certainty.

[1]) In this connection it must be pointed out that the term "0" does not mean absolutely none, but only that the number is too small to be determined by the method used.

Table 2

Plate counts on beech mor soils

Plating medium	Locality	Date of sampling	Soil moisture % of d. m.	Soil pH	Plate counts mill. per gm of dry soil	Yeasts	
						percentage	thousands per gm of dry soil
Thornton's agar	II	13. 4. 56	104	4.5	4.4	0	0
	IV	3. 5. 56	104	4.7	3.6	0	0
	V	3. 5. 56	117	—	7.5	0	0
	VIII	6. 4. 57	163	4.4	1.8	0	0
	IX	6. 4. 57	213	4.3	3.1	2.0	62
Soil extract agar	II	13. 4. 56	104	4.5	2.9	1.0	29
	IV	3. 5. 56	104	4.7	4.0	9.0	360
	V	3. 5. 56	117	—	8.4	6.0	504
	VIII	6. 4. 57	163	4.4	2.0	1.6	32
	IX	6. 4. 57	213	4.3	4.3	0	0
	V	5. 5. 58	122	4.5	25.9	1.8	466
	II	3. 7. 58	113	3.8	8.2	2.0	164
	IV	2. 12. 58	144	4.2	5.1	0	0
Trypt. gluc. extract agar	II	13. 4. 56	104	4.5	13.1	2.0	262
	IV	3. 5. 56	104	4.7	7.0	3.0	210
	V	3. 5. 56	117	—	7.5	5.0	375
	VIII	6. 4. 57	163	4.4	4.7	2.0	94
	IX	6. 4. 57	213	4.3	15.1	0	0
DPYA	II	17. 3. 61	151	4.1	2.3	1.0	23
	—	2. 6. 61	146	4.1	2.0	2.0	40
	—	8. 8. 61	149	3.9	3.1	0	0
	—	27. 9. 61	162	3.8	4.7	2.0	94
	—	5. 12. 61	138	3.8	2.6	2.0	52
	—	1. 2. 62	109	3.9	2.6	9.0	234
	IV	23. 3. 61	255	4.1	2.2	0	0
	—	29. 5. 61	81	4.2	1.3	9.0	117
	—	11. 8. 61	124	3.8	1.7	2.0	34
	—	28. 9. 61	133	3.9	1.7	11.0	187
	—	8. 12. 61	110	4.0	2.3	20.0	460
	—	2. 2. 62	135	4.0	1.3	10.0	130

In spite of this, the numbers are generally much higher than those found by previous investigators. Soils of a similar nature have been studied by LUND (1954), who found up to 16.000 yeast cells per gm. of dry soil in a Danish beech mull soil, and less than 10 cells per gm in a beech mor soil. Both localities were sampled twice, in March and in August. The yeast content of forest soils have been studied also by MILLER and WEBB (1954), who found 1000 to 2500 yeast cells per gm. of soil, and by DI MENNA (1960), who found 1000 to 8000 per gm.

This discrepancy is probably due to the different methods used, especially the different composition of the plating media. All three investigators mentioned used plating media, which were made selective for yeasts by acidification or by addition of various inhibitors (sodium propionate, rose bengal, ox-gall), whereas the counts recorded here were made chiefly on non-selective media. It is true that DPYA contains both sodium propionate and ox-gall, but only in half or less than half of the concentrations used by LUND (1954) and MILLER and WEBB (1954), and yet the depressing effect of these additions is manifest.

The separation of yeasts from filamentous fungi by means of selective media constitutes a very difficult problem because of the nutritional and biochemical similarity between the two groups. Presumably it is impossible to inhibit growth of filamentous fungi without also suppressing some of the yeasts.

As regards the occurrence of yeasts at the different seasons of the year, the DPYA series indicates that the highest numbers of yeasts are found in autumn and winter, and the lowest numbers in spring and summer. However, the results are too few in number to permit dependable conclusions. Neither is it possible to demonstrate any influence of soil moisture or of soil pH on the number of yeasts.

Enrichment culture experiments

As a supplement to the plate counts a series of enrichment culture experiments was made.

The enrichment medium was a solution of 2 per cent commercial malt extract in tap water. After autoclaving 3 mgm streptomycin and 3 mgm aureomycin per 100 ml were added to the solution. The medium was distributed in 300 ml Erlenmeyer flasks with 100 ml in each, and each flask was inoculated with 1 gm of soil.

In cultures prepared in this manner growth of bacteria is inhibited almost completely, and extensive growth of filamentous fungi was prevented by cautious shaking of the flasks now and then as described by HESSELTINE et al. (1952). Yeasts, possibly occurring, were therefore detected easily, and the subsequent isolation by plating on malt extract agar was accomplished without difficulty.

The soil samples used in these experiments were drawn from the localities I, II, III and IV on three occasions, in April-May, at the end of June, and in the beginning of September 1960, and all samples without exception were found to contain yeasts or yeast-like fungi. A total of 28 strains were isolated for further examinations.

Enrichment cultures on nitrogen-deficient media inoculated with soil from the same 4 localities have also been studied (V. JENSEN 1962), and a characteristic yeast species was found commonly in these cultures, irrespective of the soil type. Isolation was carried out only from one of the cultures, however, and two strains were isolated.

Studies on the occurrence of yeasts in soil by means of enrichment cultures have been carried out on an extensive scale. Reviews on the literature on this subject can be found e. g. in the works of MRAK and PHAFF (1948) and LUND (1954).

Studying this literature it seems remarkable how the number of yeasts found in the soil has increased in the course of time. Early investigators found yeasts only sporadically in soil, mostly in orchards, and considered the soil only as a reservoir for yeasts. In later investigations, however, the presence of yeasts has

been demonstrated in practically all samples studied. BOUTHILET (1953) found yeasts in 100 per cent of 84 samples, and LUND (1954) in 90 per cent of 136 samples.

The reasons for this change are probably two: the wider definition of the yeast group, now generally accepted, and an ameliorated technique for detection and isolation of yeasts. The general conclusion to be drawn is that yeasts and yeast-like fungi in the current conception of the term, are widely distributed in the soil, and that almost every soil contains one or more species belonging to this group.

Descriptions of the isolated yeast strains

A total of 171 strains were isolated from plate counts and 30 strains from enrichment cultures. The purity of these cultures was secured by platings on malt extract agar and by frequent microscopic examinations. In a few cases addition of antibiotics to the plating medium was necessary to get rid of bacterial contaminants.

The morphological characteristics were studied on malt extract agar (2 per cent commercial malt extract, 0.2 per cent neopeptone [Bacto], and 2 per cent agar in tap water), and on Wickerham's yeast morphology agar (Bacto). Both slide cultures as described by LODDER and VAN RIJ (1952) and Dalmau plates as described by WICKERHAM (1951) have been used to study pseudomycelium formation.

Gorodkowa's agar and the acetate agar of Kleyn (WINDISCH 1960) have been used to promote the formation of ascospores. However, the only strains, in which ascospore formation was detected, formed the spores freely, also on other media, e. g. malt extract agar.

The fermentation of carbohydrates was studied in large Durham tubes in a solution of 1 per cent yeast extract (Bacto) and 2 per cent carbohydrate in distilled water, pH c. 7. First, fermentation of glucose was tested on all strains, and then the glucose-fermenting strains were tested for fermentation of galactose, saccharose, maltose, lactose and raffinose.

The assimilation of carbon compounds was studied by means of Wickerham's yeast nitrogen base (Bacto). In most cases tests were performed only with the five sugars used by LODDER and VAN RIJ (1952) (glucose, galactose, saccharose, maltose and lactose) and ethanol. Utilization of nitrate was tested by means of Wickerham's yeast carbon base (Bacto).

Splitting of arbutin and aesculin was tested as described by LODDER and VAN RIJ (1952) for arbutin, and formation of lipase was examined by the method of SIERRA (1957). Production of starch was tested as described by WICKERHAM (1951) on the carbon assimilation cultures.

Experiments have been made with a number of other tests (action on litmus milk, growth on potato slices, hydrolysis of starch, gelatin and casein), but the results were usually negative or uncertain and difficult to interpret. They seemed of but little importance and therefore are not recorded here.

Group no. 1, *Saccharomyces fructuum* (1 strain)

The malt agar streak is white or slightly grey, soft and smooth, dull to shining. In malt extract abundant sediment, and usually a ring, are formed.

The cells are spherical to short-oval 3—5 μ × 4—7 μ, often with a large refractive globule. No pseudomycelium is formed.

Ascospores are formed readily on malt extract agar, 2—4 per ascus. They are spherical, 2—3 μ in diameter, with smooth surface, and contain a small refractive granule (fig. 1). Conjugation follows immediately after germination of the ascospores.

Glucose, galactose, saccharose and raffinose are fermented, but not maltose or lactose.

Glucose, galactose, saccharose, maltose and ethanol are assimilated, but not lactose.

Nitrate is not utilized.
Splitting of arbutin and aesculin: negative.
Lipase is not formed.
Starch is not produced.

This strain belongs to the genus *Saccharomyces* (Meyen) Reess, and the description agrees in most details with the standard description of *S. fructuum* LODDER and VAN RIJ.

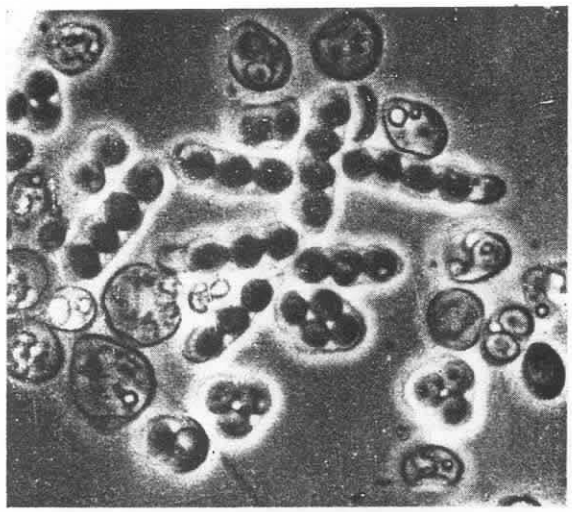


Fig. 1.
Saccharomyces fructuum. Malt agar culture with asci. Phase. 1400 ×

Group no. 2, *Pichia* sp. 1 (1 strain)

The streak culture on malt extract agar is whitish, in older cultures cream-coloured, smooth and glistening. In malt extract abundant sediment is formed, and a slight pellicle formation may be observed.

The cells are regular, oval, 2—4 μ × 3—7 μ, and usually contain a few small, refractive globules (fig. 2). Ascospores are formed readily on malt extract agar, 1—4 per ascus. They

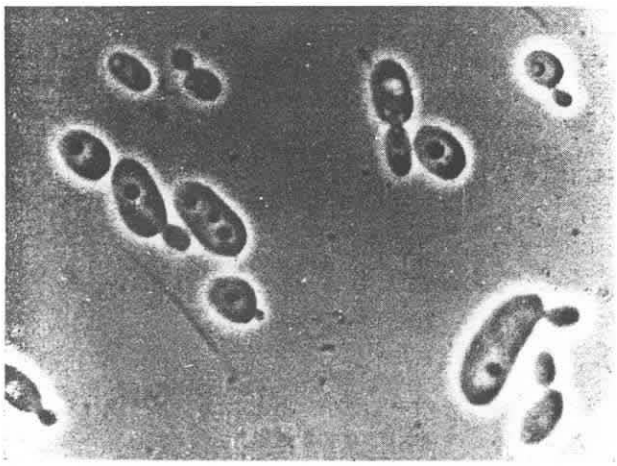


Fig. 2.
Pichia sp. 1. Malt agar culture, 2 days old. Phase. 1200 ×

are hat-shaped and contain a single, refractive globule (fig. 3). A rather well-developed pseudomycelium is formed in slide cultures.

Carbohydrates are not fermented.

Glucose, saccharose, maltose and ethanol are assimilated, but not galactose, lactose and raffinose.

Nitrate is not utilized.
Splitting of arbutin and aesculin: positive but weak.
Lipase is formed.

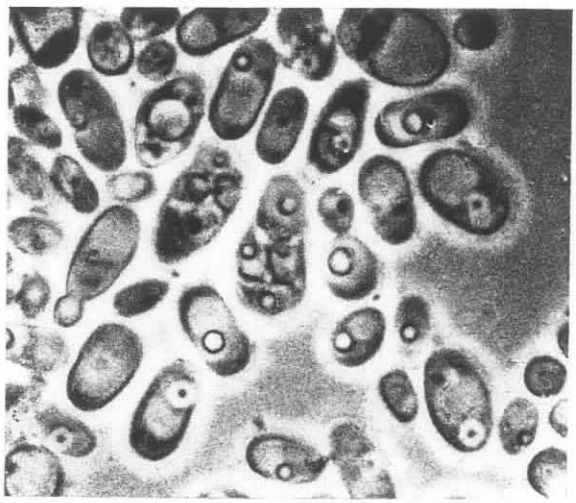


Fig. 3.
Pichia sp. 1. Older malt agar culture. In the centre and ascus with 4 hat-shaped spores. Phase. 1800 ×

The systematic position of this strain must be within the genus *Pichia* Hansen, as amended by PHAFF (1956). However, the description does not agree with any of the existing standard descriptions. It seems to be most closely related to *Pichia rhodanensis* (Ramirez et Boidin) Phaff but it differs from this species primarily in the lack of glucose fermentation.

Group no. 3, *Lipomyces starkeyi* (2 strains)

The streak culture on malt extract agar is very mucous and glistening, whitish and semi-translucent. In malt extract a sediment and a ring are formed, but no pellicle.

The cells are spherical to short-oval 4—6 μ × 5—8 μ, and surrounded by large slime capsules. The cells are very rich in fat, which often almost fills the entire cell, either as one

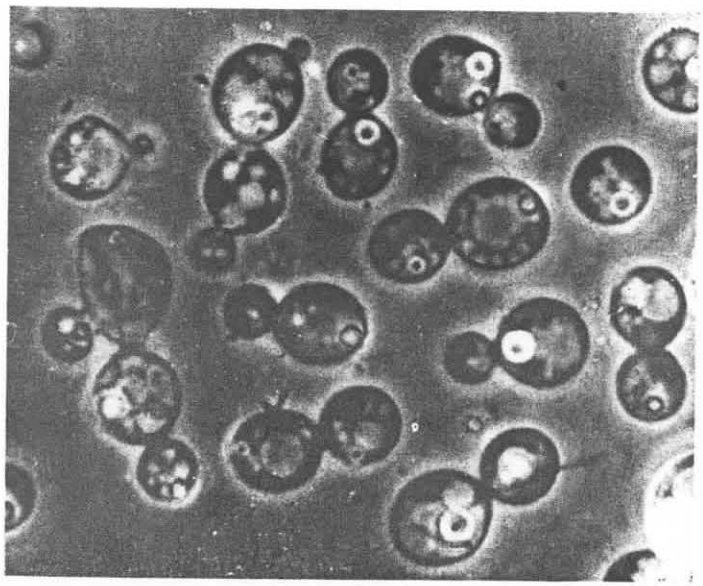


Fig. 4.
Lipomyces starkeyi. Malt agar culture, 2 days old. Phase. 1400 ×

large or as many small globules (fig. 4). In older cultures irregular, sac-like protuberances are formed. No pseudomycelium.

Ascospores are formed readily on malt extract agar by both strains, with up to 16 spores per ascus (fig. 5). The spores are spherical, 2—3 μ in diameter. They may be formed within normal cells or within the sac-like protuberances.

Carbohydrates are not fermented.

Glucose, galactose, saccharose and maltose are utilized well, ethanol only slightly. Lactose is not assimilated.

Nitrate is not utilized.

Splitting of arbutin and aesculin: positive, but weak.

Lipase is not formed.

The properties of these two strains agree in practically all details with the standard description of *Lipomyces starkeyi* Lodder et van Rij.

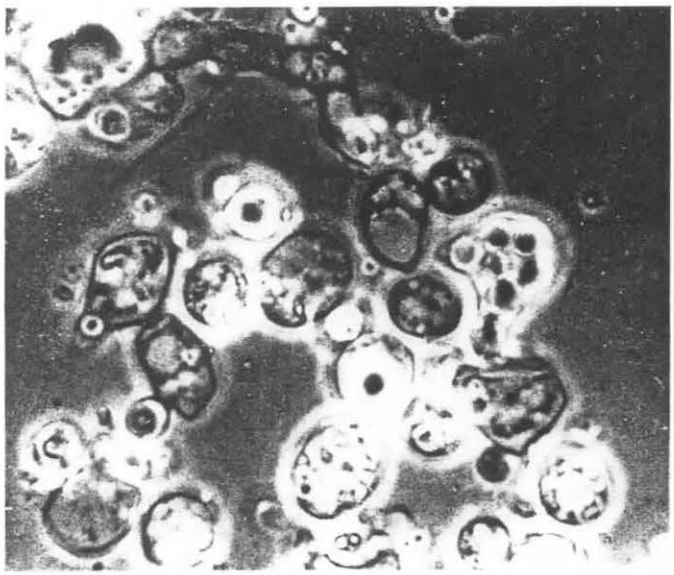


Fig. 5.
Lipomyces starkeyi. Older malt agar culture. To the right an ascus with c. 8 spores. Phase, 1400 ×

Group no. 4, *Cryptococcus laurentii* (5 strains)

The malt agar streak is semi-translucent, whitish, smooth, glistening and very mucous; older cultures are pale brown. In malt extract, abundant sediment and a ring or a thin pellicle are formed.

The cells are oval to long-oval, c. 3 μ × 4—6 μ, and surrounded by well-developed slime capsules. No pseudomycelium or only a rudimentary one is formed, and no ascospores.

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.

Nitrate is not utilized.

Splitting of arbutin: negative.

Splitting of aesculin: positive.

Lipase is formed.

Starch is produced.

This description agrees fairly well with the standard description of *Cryptococcus laurentii* (Kufferath) Skinner, and in all probability the five strains belong to this species.

Group no. 5, *Cryptococcus albidus* (4 strains)

The malt agar streak is whitish to cream-coloured, glistening, and of a more or less mucous consistency, sometimes almost fluid. In malt extract, abundant sediment, a ring and occasionally some islets are formed.

The cells are spherical to short-oval, 2.5—5 μ × 4—6 μ, usually with a finely granulated protoplasm, and surrounded by well-developed slime capsules (fig. 6). No pseudomycelium and no ascospores are formed.

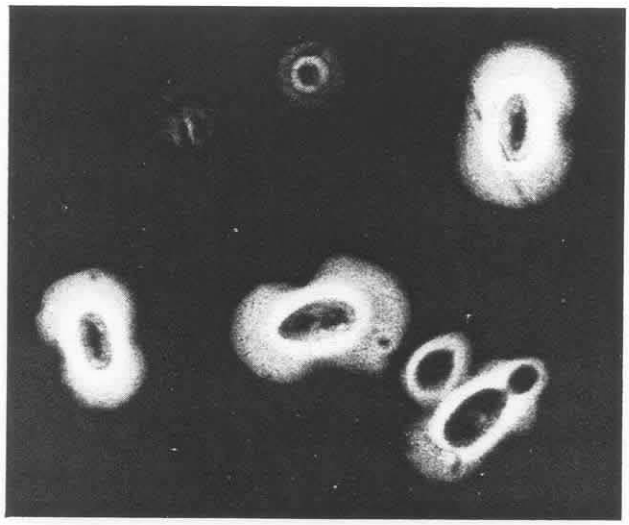


Fig. 6.
Cryptococcus albidus. Wet Indian ink preparation. Phase. 1600 ×

Carbohydrates are not fermented.
Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.
Nitrate is utilized.
Splitting of arbutin and aesculin: positive, but sometimes weak.
Lipase is formed.
Starch is produced.

These strains presumably belong to the species *Cryptococcus albidus* (Saito) Skinner. The properties agree in almost all details with the standard description of this species.

Group no. 6, *Torulopsis* sp. 1 (1 strain)

The malt agar streak is grey, flat and dull. In malt extract a sediment and a thin, grey, creeping pellicle are formed.

The cells are spherical to short-oval, or more irregular, 3—5 μ × 4—10 μ, and contain one or occasionally two, large refractive globules. No pseudomycelium and no ascospores are formed.

Glucose and saccharose are fermented readily. Fermentation of galactose, maltose and raffinose is weaker and delayed. Lactose is not fermented.

Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.
Nitrate is not utilized.
Splitting of arbutin and aesculin: positive.
Lipase is not formed.
Starch is not produced.

This strain must belong to the genus *Torulopsis* Berlese, but it differs clearly from all species described by LODDER and VAN REJ (1952) and from all other species known to the author.

Group no. 7, *Torulopsis famata* (4 strains)

The malt agar streak is white, filiform, smooth and glistening. In malt extract abundant sediment and a thin, dry pellicle are formed.

The cells are small, 2—3 μ × 3—5 μ, short-oval to oval or more irregular (fig. 7). No pseudomycelium is formed or only rudimentary. No ascospores.

The fermentation reactions are weak and delayed. 3 strains ferment glucose and galactose. The fourth strain ferments only glucose, but nevertheless it is included in this group, because in all other characters it is identical with the other three strains.

Glucose, galactose, saccharose, maltose and ethanol are assimilated, but not lactose.

Nitrate is not utilized.

Splitting of arbutin and aesculin: positive, but sometimes weak.

Lipase is formed.

Starch is not produced.

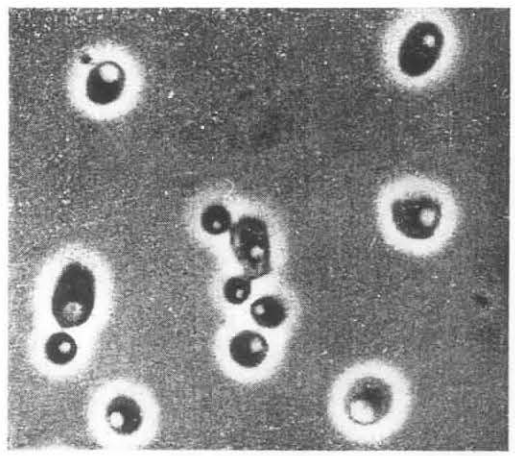
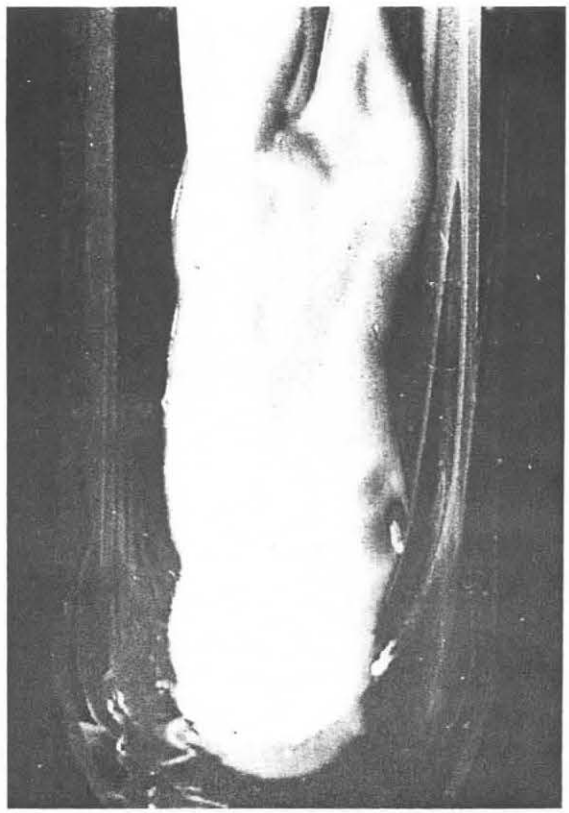


Fig. 7.
Torulopsis famata. Malt agar culture, 2 days old. Phase, 1600 ×

Also this group must be classified within the genus *Torulopsis* Berlese, and the four strains seem to be closely related to *T. famata* (Harrison) Lodder et van Rij. They differ from this species only by the fermentation of galactose and the pellicle formation. LUND (1952), however, mentions strains, supposed to belong to *T. famata*, which formed pellicles in old cultures

Group no. 8, *Torulopsis aeria* (21 strains)



The malt agar streak is white to cream-coloured, soft, smooth and glistening (fig. 8). In malt extract abundant sediment and a ring are formed, but no pellicle.

The cells are spherical to short-oval, 3—5 μ in diameter, and usually they contain a large fat globule, which sometimes almost fills the entire cell (fig. 9). Only a very primitive pseudomycelium is formed, or none at all. No ascospores.

Carbohydrates are not fermented

Glucose, galactose, saccharose, maltose and lactose are assimilated. Growth with ethanol as carbon source is variable, usually weak.

Nitrate is utilized.

Splitting of arbutin: negative.

Splitting of aesculin: positive.

Lipase is not formed.

Starch is not produced.

Fig. 8.
Torulopsis aeria. Malt agar culture, 2.5 ×

This group probably belongs to the species *Torulopsis aeria* (Saito) Lodder. Both morphological and physiological properties are in fairly good agreement with the standard description of this species.

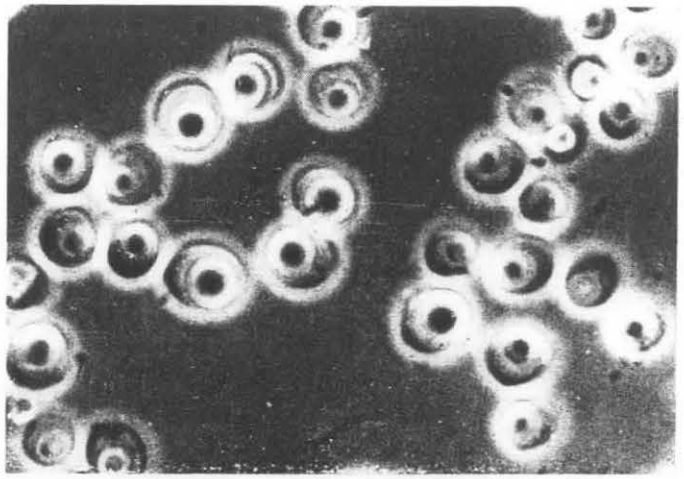


Fig. 9.
Torulopsis aeria. Malt agar culture, 2 days old. Phase. 1500 ×

Group no. 9. *Torulopsis ingeniosa* (1 strain)

The malt agar streak is whitish, smooth and glistening. In malt extract a sediment, a ring and occasionally some islets are formed.

The cells are oval to long-oval, 2—4 μ × 4—10 μ, sometimes occurring in short chains. No pseudomycelium is formed, and no ascospores.

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.

Nitrate is utilized.

Splitting of arbutin and aesculin: negative.

Lipase is not formed.

Starch is not produced.

This strain presumably is identical with *Torulopsis ingeniosa* di Menna (1958), although a slime capsule has not been demonstrated with certainty. All other characters are in good agreement with the standard description of this species.

Group no. 10. *Torulopsis candida* (1 strain)

The malt agar streak is cream-coloured, smooth and flat. Older cultures are pale brown and dull. In malt extract a sediment, a ring and occasionally some islets are formed.

The cells are almost spherical, 3—6 μ in diameter, usually with a large fat globule (fig. 10). No pseudomycelium is formed, and no ascospores.

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose and lactose are assimilated. Weak growth with ethanol as carbon source.

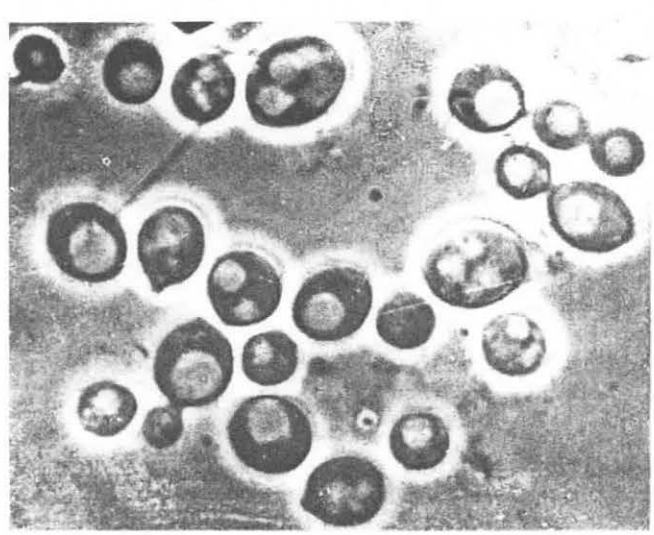


Fig. 10.
Torulopsis candida. Malt agar culture, 2 days old. Phase. 1500 ×

Nitrate is not utilized.
Splitting of arbutin: positive.
Starch is not produced.

The characters of this strain agree in practically all details with the standard description of *Torulopsis candida* (Saito)Lodder.

Group no. 11, *Torulopsis* sp. 2 (1 strain)

The malt agar streak is grey to cream-coloured, soft and smooth to dull. In malt extract a sediment and a ring are formed, but no pellicle.

The cells are oval to oblong and often lemonshaped with polar or bipolar budding, 3—5 μ × 5—10 μ. They may occur in pairs or short chains. No pseudomycelium and no ascospores are formed.

Carbohydrates are not fermented.
Glucose, galactose, saccharose, maltose and ethanol are assimilated, but not lactose.
Nitrate is utilized.
Splitting of arbutin: negative.
Splitting of aesculin: positive.
Lipase is not formed.
Starch is not produced.

The morphology of this strain suggests a relationship to the genus *Kloeckera* Janke, but since it is unable to ferment carbohydrates and utilizes nitrate, it must be placed within the genus *Torulopsis* Berlese. It is not identical with any species hitherto described.

Group no. 12, *Candida solani* (1 strain)

The malt agar streak is white to cream-coloured, with a more or less wrinkled surface and a fringe of pseudomycelium (fig. 11). In malt extract abundant sediment and a thick pellicle are formed.

The cells are variable, round to oval or more irregular, 3—5 μ in diameter (fig. 12). A typical pseudomycelium is formed, but no ascospores.

Only glucose is fermented.
Glucose, saccharose and maltose are utilized well, galactose and ethanol only slightly. Lactose is not utilized.
Nitrate is not utilized.
Splitting of arbutin and aesculin: positive.
Lipase is not formed.
Starch is not produced.

This strain probably belongs to the species *Candida solani* Lodder et van Rij, although there are some discrepancies in morphology, and no fermentation of saccharose could be detected. According to LODDER and VAN RIJ (1952) saccharose is fermented only very weakly by *C. solani*.

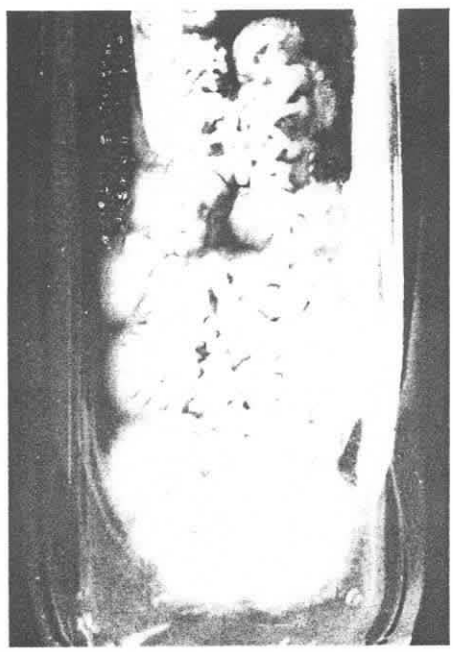


Fig. 11.
Candida solani. Malt agar culture, 2.5 ×.

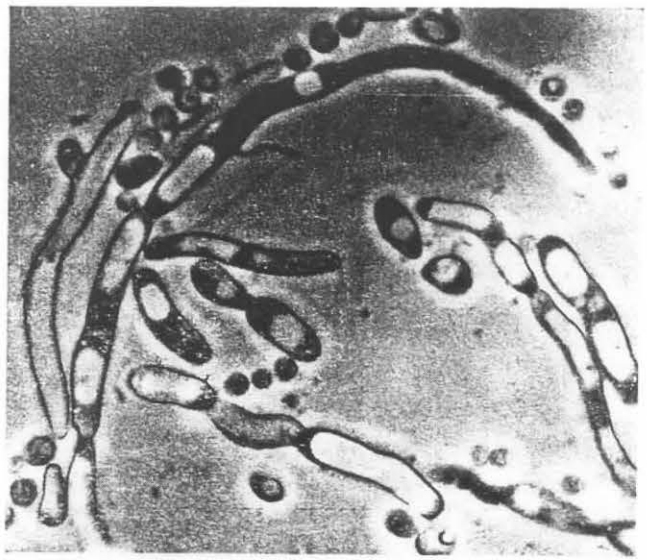


Fig. 12.
Candida solani. Malt agar culture, 2 days old. Phase, 1400 ×

Group no. 13, *Candida krusei* (3 strains)

Growth on malt extract agar is rather scant. The streak culture on yeast morphology agar is white, wrinkled and folded in the middle, with a smooth margin and a fringe of pseudomycelium. In malt extract a sediment and some islets are formed.

The cells are oval to oblong or more irregular, 3—4 μ in diameter (fig. 13). A well-developed pseudomycelium is formed, but no ascospores.

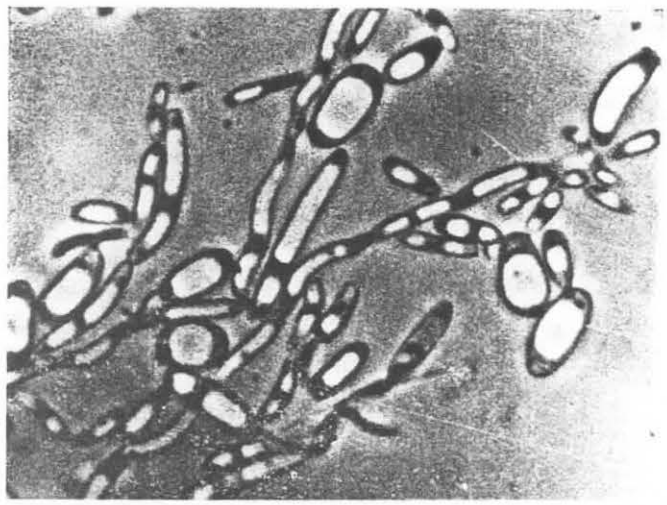


Fig. 13.
Candida krusei. Malt agar culture, 2 days old. Phase. 1400 ×

Only glucose is fermented.

Glucose is utilized well, ethanol only slightly. Saccharose, maltose and lactose are not assimilated. Galactose is assimilated only by one strain.

Nitrate is not utilized.

Splitting of arbutin and aesculin: positive.

Lipase is not formed.

Starch is not produced.

Morphologically these strains are very similar to *Candida krusei* Cast. Berkhout. The physiological properties also agree fairly well with the standard description of this species.

except for the splitting of arbutin, which is described as negative by LODDER and VAN RIJ (1952). One strain differs also in assimilation of galactose.

Group no. 14, *Candida humicola* (27 strains)

The malt agar streak is whitish to grey, dull, flat or more or less wrinkled or folded, and usually with a marked fringe of pseudomycelium (fig. 14). In malt extract a sediment and a thick, grey, dull and often folded pellicle are formed.

The cells are very variable both in size and shape, usually 3—5 μ in diameter (fig. 15 and 16). A well-developed pseudomycelium is formed with long and slender cells, often approaching a true mycelium. No ascospores are formed.

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.

Nitrate is not utilized.

Splitting of arbutin: weak or negative.

Splitting of aesculin: usually positive.

Lipase is formed.

Production of starch was detected in a few strains.

This group belongs to the genus *Candida* Berkhout, and most probably should be identified as *C. humicola* (Daszewska) Diddens et Lodder. The properties are in good agreement with the standard description of this species, except for the weak action on arbutin. According to LODDER and DAN RIJ (1952) splitting of arbutin should be positive in *C. humicola*.

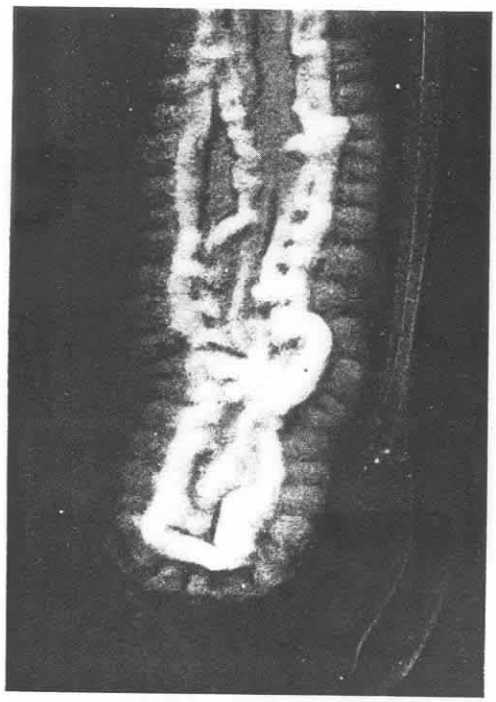


Fig. 14.
Candida humicola. Malt agar culture. 2.5 ×

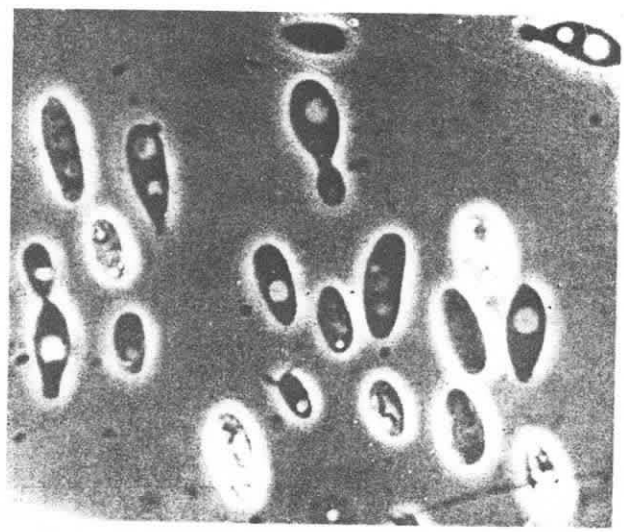


Fig. 15.
A strain of *Candida humicola*. Malt agar culture, 2 days old. Phase. 1500 ×

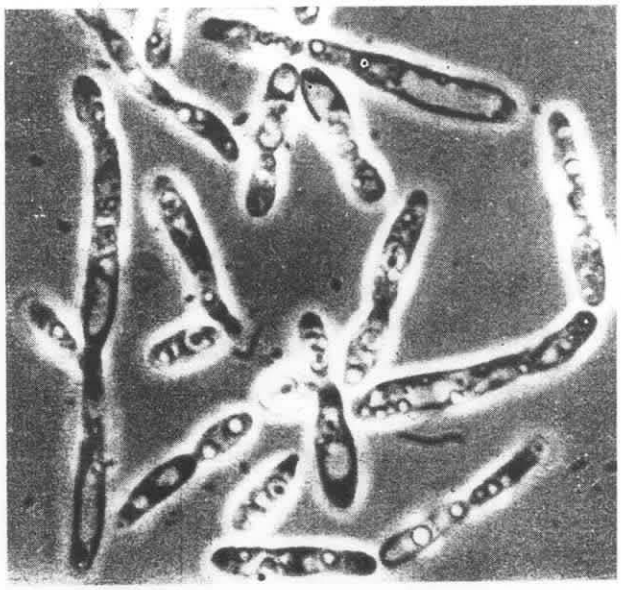


Fig. 16.
A strain of *Candida humicola*. Malt agar culture, 2 days old. Phase. 1400 ×

Group no. 15, *Candida curvata* (2 strains)

The malt agar streak is cream-coloured, soft, raised and occasionally hairy, with a more or less wrinkled or folded surface and a marked fringe of pseudomycelium (fig. 17). In malt extract, abundant sediment and a grey, dull, creeping pellicle are formed.

The cells are very variable, 2.5—4 μ × 3.5—10 μ. The smaller cells are spherical to oval, the larger cells oblong or more irregular, often curved (fig. 18). Large fat drops and vacuoles,

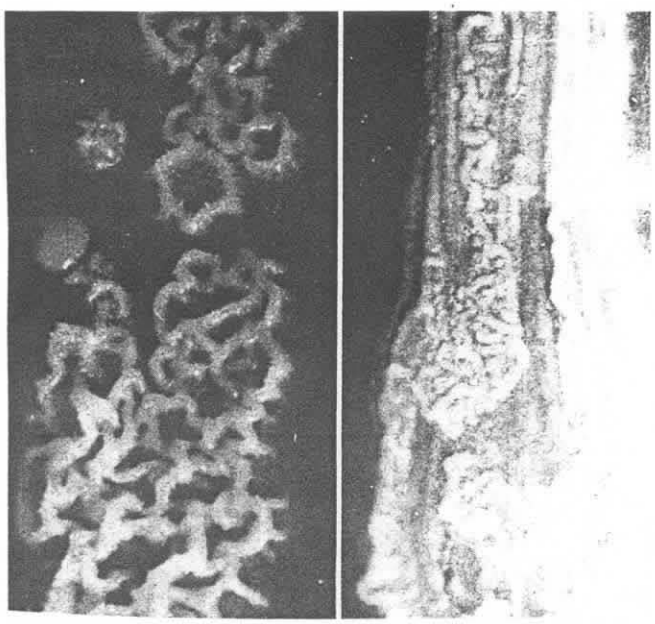


Fig. 17.
Two different strains of *Candida curvata*. Malt agar cultures. 2.5 ×

are often observed. A well-developed pseudomycelium is formed, often with very long cells, resembling a true mycelium. No ascospores are formed.

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.

Utilization of nitrate: absent or very weak.
Splitting of arbutin and aesculin: positive.
Lipase is formed.
Starch is not produced.
Litmus milk is coagulated and cleared.

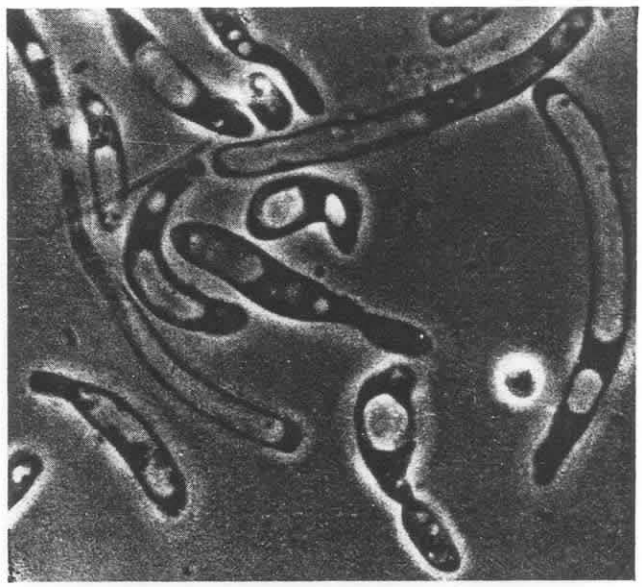


Fig. 18.
Candida curvata. Malt agar culture, 2 days old. Phase. 1400 ×

These two strains are probably identical with the species *Candida curvata* (Diddens et Lodder) Lodder et van Rij. Both morphological and physiological properties are in good agreement with the standard description of this species.

Group no. 16, *Candida* sp. 1 (81 strains)

The malt agar streak is grey to cream-coloured, soft and smooth with a narrow fringe of pseudomycelium. Older cultures assume a dirty yellow to orange colour, with a dull and

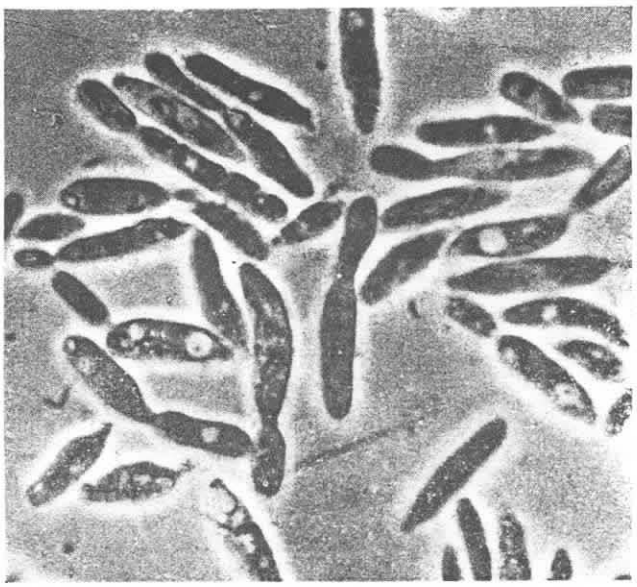


Fig. 19.
Candida sp. 1. Cells grown on yeast morphology agar, 2 days old. Phase. 1500 ×

sometimes wrinkled or folded surface. In malt extract a sediment and a ring or a more or less well-developed pellicle are formed.

The cells are variable and irregular, often oblong and pointed, pyriform or fusiform, 2—4 μ in diameter. Usually the protoplasm is granulated, and sometimes concentrated in one end of the cell (fig. 19 and 20). A pseudomycelium is formed, but it is often rather primitive, merely consisting of chains of cells. No ascospores are formed.

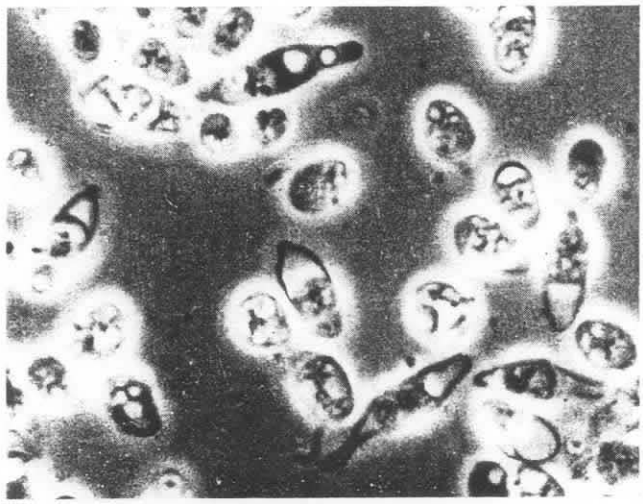


Fig. 20.
Candida sp. 1. Malt agar culture. 2 days old. Phase, 1600 ×

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose and lactose are assimilated. Weak growth with ethanol as carbon source.

Nitrate is utilized.

Splitting of arbutin and aesculin: positive, but often weak.

Lipase is formed.

Starch is not produced.

This group belongs to the genus *Candida* Berkhout, but the properties do not agree with any of the descriptions given by LODDER and VAN RIJ (1952) or with any other species description known to the author.

Group no. 17, *Candida* sp. 2 (2 strains)

The malt agar streak is whitish, smooth to dull, soft and mucous, with a fringe of pseudomycelium. In malt extract a sediment and a thin, creeping pellicle are formed.

The cells are oblong or more irregular, 2—3 μ × 3—8 μ, with granular protoplasm. Pseudomycelium is richly developed, but no ascospores are formed.

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.

Nitrate is utilized.

Splitting of arbutin and aesculin: positive.

Lipase is formed.

Starch is not produced.

These two strains belong to the genus *Candida* Berkhout. They are closely related to the preceding group, but differ clearly by the macroscopical appearance of colonies and cultures.

Group no. 18, *Candida* sp. 3 (19 strains)

The malt agar streak is white to cream-coloured, raised and folded, with a glistening surface, and surrounded by a very marked mycelial zone (fig. 21). In malt extract a sediment and a thick, dry, white, folded pellicle are formed.

The cells are very variable both in size and shape, usually 3—5 μ in diameter (fig. 22).

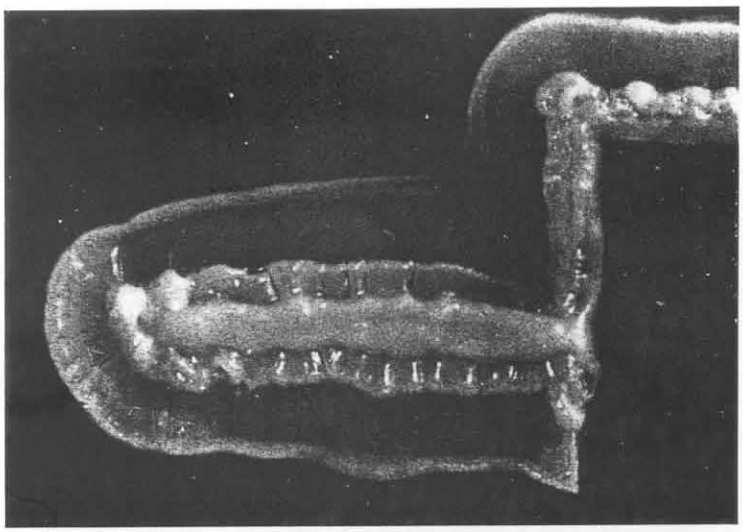


Fig. 21.
Candida sp. 3. Dalmau plate with yeast morphology agar, 2.5 ×

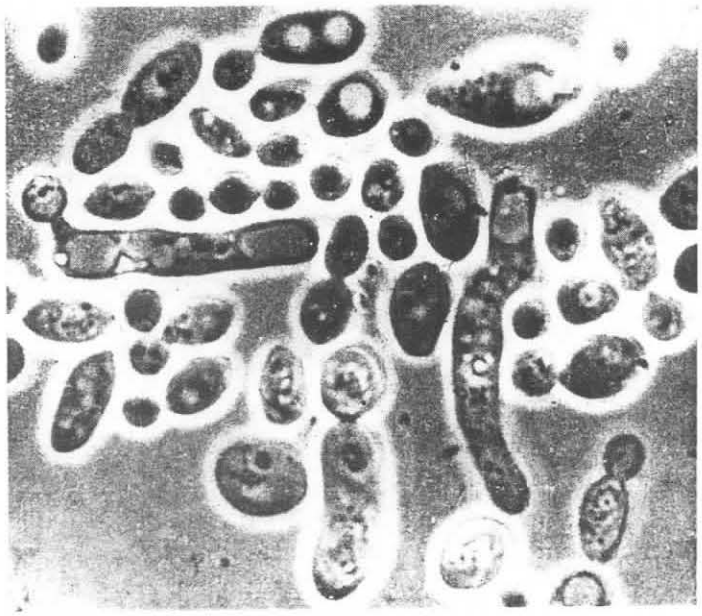


Fig. 22.
Candida sp. 3. Malt agar culture, 2 days old. Phase. 1200 ×

A well-developed pseudomycelium is formed with very long and slender cells, approaching a true mycelium, but arthrospores have not been observed. No ascospores are formed.

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.

Nitrate is utilized.

Splitting of arbutin and aesculin: positive.

Lipase is formed.

Starch is not produced.

Also this group must belong to the genus *Candida* Berkhout, but it is not identical with any species, hitherto described.

Group no. 19, *Trichosporon* sp. 1 (19 strains)

The growth on malt extract agar is rather scant. The streak culture is whitish to grey, thin and flat, often of a tough or cartilaginous consisteney. On yeast morphology agar, growth

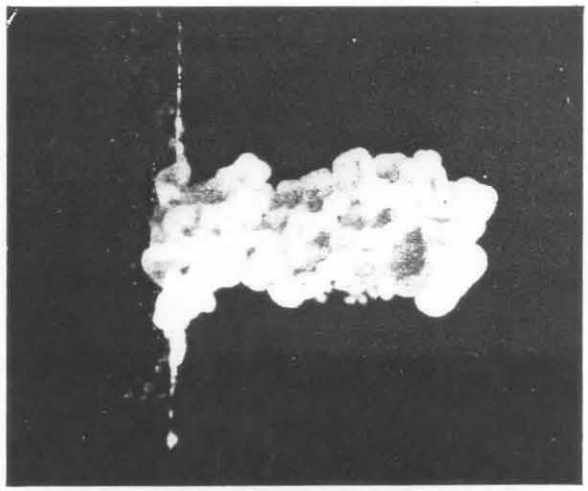


Fig. 23.
Trichosporon sp. 1. Dalmau plate with yeast morphology agar. 3.5 ×

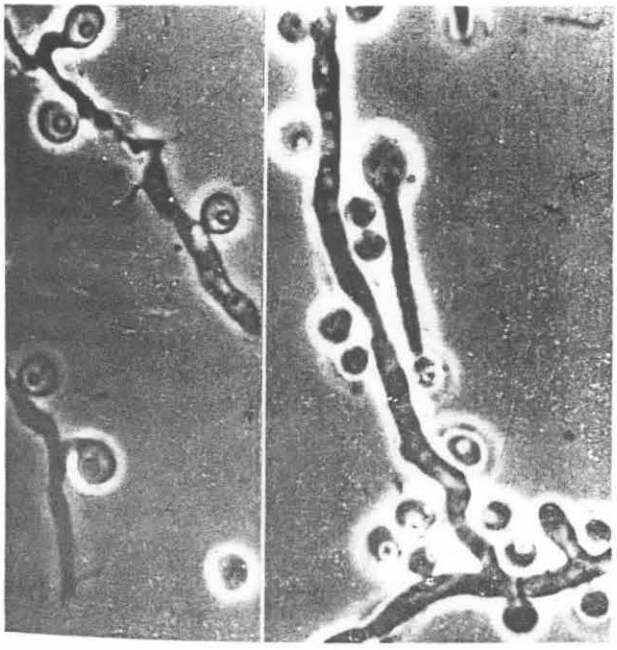


Fig. 24.
Trichosporon sp. 1. Malt agar culture, 2 days old. Phase. 1200 ×

is more abundant. The streak is whitish, raised, with irregular margin and strongly folded surface (fig. 23). In malt extract a flocculent sediment and sporadical surface growth in the shape of an incoherent ring and small islets are formed.

Both pseudomycelium and true mycelium are formed, but the latter predominates. Blastospores are formed on the mycelium, single or in small clusters (fig. 24). They are spherical to

short-oval, c. 3 μ in diameter, and usually contain a refractive globule. Arthrospores could not be demonstrated with certainty. No ascospores are formed.

Carbohydrates are not fermented.

Glucose, galactose, saccharose and maltose are utilized well, lactose and ethanol only weakly.

Nitrate is utilized.

Splitting of arbutin and aesculin: weak or negative.

Lipase is not formed.

Starch is not produced.

The systematic position of these strains could not be determined with certainty, but the preponderance of true mycelium suggests a relationship to the genus *Trichosporon* Behrend, although formation of arthrospores could not be detected.

Group no. 20, *Rhodotorula mucilaginosa* (3 strains)

The streak culture on malt extract agar is filiform, smooth and often glistening, with a pink to red pigmentation. In malt extract a sediment, a ring and later a thin pellicle are formed.

The cells are uniform and regularly oval, 2—4 μ × 3—5 μ (fig. 25). No pseudomycelium and no ascospores are formed.

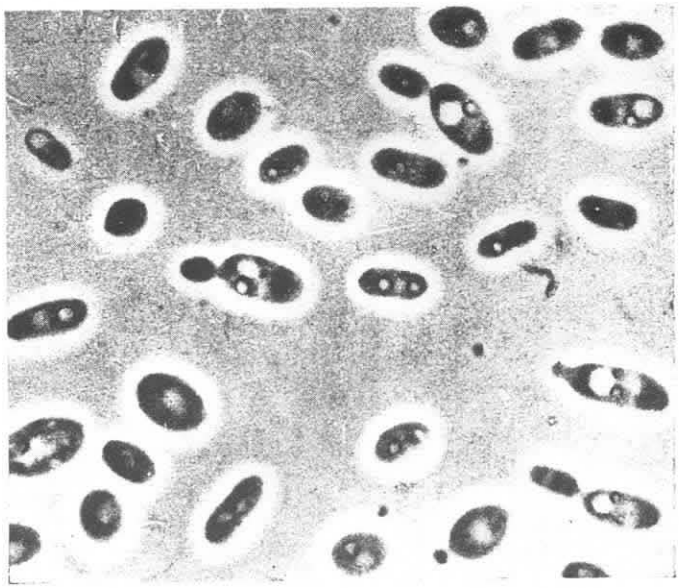


Fig. 25.
Rhodotorula mucilaginosa. Malt agar culture, 2 days old. Phase. 1200 ×

Carbohydrates are not fermented.

Glucose, saccharose and maltose are utilized well, galactose only weakly. Lactose and raffinose are not assimilated.

Nitrate is not utilized.

Splitting of arbutin and aesculin: positive.

Lipase is formed.

The properties of these strains are in good agreement with the standard description of *Rhodotorula mucilaginosa* (Jørg.) Harrison. According to Lodder and van Rij (1952) only a ring should be formed in malt extract, but Lund (1954) mentions a strain of *R. mucilaginosa*, which also formed a pellicle.

Group no. 21, *Rhodotorula* sp. 1 (1 strain)

The malt agar streak is soft and smooth, grey at first, but later distinctly red. Older cultures are dull and red. In malt extract abundant sediment and a ring are formed.

The cells are mainly spherical to oval of very variable size, but more irregular cells are also found. No pseudomycelium and no ascospores are formed.

Carbohydrates are not fermented.

Glucose, galactose, saccharose, maltose, lactose and ethanol are assimilated.

Nitrate is utilized.

Splitting of arbutin: negative.

Also this strain must be classified within the genus *Rhodotorula* Harrison, but it is not identical with any of the species hitherto described.

Group no. 22, systematic position unknown (1 strain)

The malt agar streak is grey to cream-coloured, dull, with a more or less wrinkled surface and a marked fringe of mycelium and pseudomycelium. On yeast morphology agar growth is very vigorous, the colonies are raised and much folded (fig. 26). In malt extract a sediment and a thin, dull, creeping pellicle are formed.

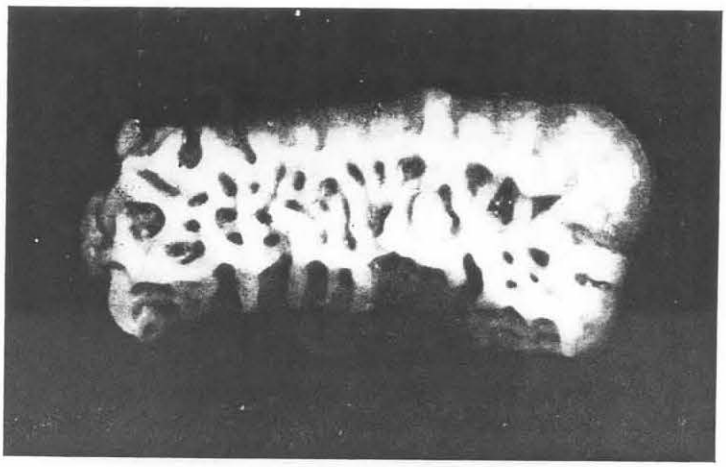


Fig. 26.
Group 22. Dalmau plate with yeast morphology agar. 3 ×

The cells are large and cylindrical with a granular protoplasm, 3—5 μ in diameter. Vegetative reproduction by fission only. When division of a cell is completed, the two daughter cells, by a sudden movement, are placed at an angle to each other (fig. 27). In older cultures the cells are short-oval or more irregular. Both pseudomycelium and true mycelium, which breaks up into arthrospores, are formed abundantly. Ascospores have not been observed.

Carbohydrates are not fermented.

Glucose, saccharose, maltose, lactose and ethanol are assimilated, but not galactose.

Nitrate is not utilized.

Splitting of arbutin: negative.

Splitting of aesculin: positive.

Lipase is formed.

The systematic position of this strain is uncertain. Morphologically it resembles the genus *Schizosaccharomyces* Lindner, especially the species *S. versatilis* Wickerham et Duprat, but neither ascosporeformation nor fermentation of carbohydrates could be demonstrated.

It will appear from the above descriptions and remarks that the identification of soil yeasts is a difficult task, and the results often uncertain and unsatisfactory. The morphology is variable and highly dependent on growth conditions, and often

changes in the course of time. It is well known e.g. that mycelium formation is more conspicuous in many cases in old stock-cultures than in recently isolated strains.

The physiological characters are more stable, but the properties used in the general standard descriptions are often insufficient to allow a positive identifi-

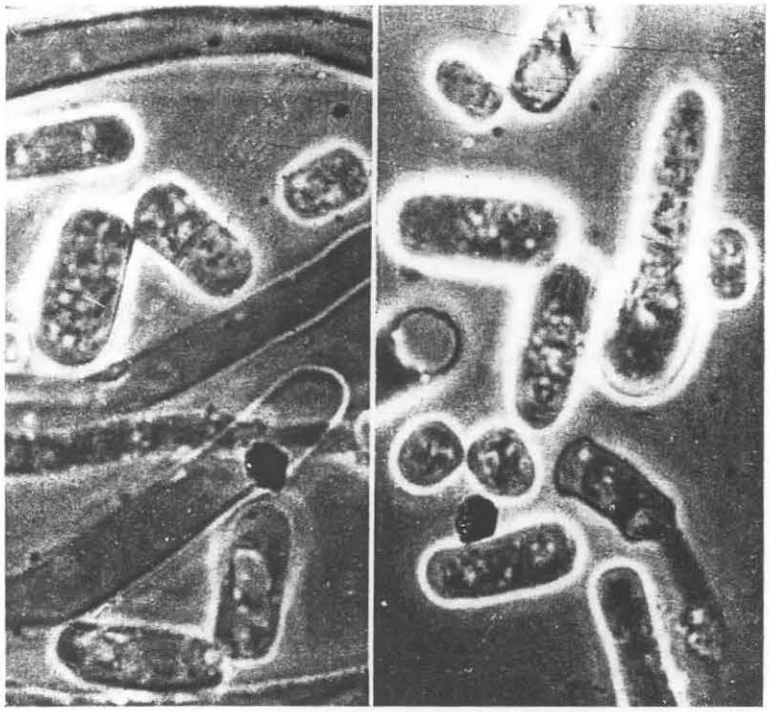


Fig. 27.
Group 22. Malt agar culture 3 days old. Phase, 1400 ×

cation, and the need for an additional number of distinguishing characters is very marked. The classic work of LODDER and VAN RIJ (1952) is extremely helpful, when taxonomic studies on yeasts are made, but a revised edition, including the large number of new species, described during the past 10 years, and with an enlarged and amended scheme of description, is greatly needed.

Distribution of the various species in mull and mor soils

In tables 3 and 4 all the yeast strains examined are tabulated, and their origin in mull or mor soils stated. It appears from the tables that there is not only a quantitative difference, but also a qualitative difference between the two soil types.

A total of 20 species have been demonstrated in mor soils (including *Lipomyces starkeyi*, cf. p. 44), but only 13 species in the mull soils. 9 species were found exclusively in mor soils, whereas only 2 species were found exclusively in mull soils, and only one species, *Candida humicola*, was definitely more common in mull than in mor soils.

The tables also show that the plate counts give a picture of the yeast population, which differs from that obtained by the enrichment technique. 12 species were found exclusively in the plate counts, and 5 species exclusively in the enrichment cultures; only 5 species were found by both methods.

The species found in the plate counts are the typical soil yeasts, occurring in large numbers in the soil, whereas those found only by the enrichment technique well may be widespread in the soil, but always in very small numbers.

The enrichment cultures are so prepared as to be suitable for the typical yeasts, and when such species occur, they will be able to supersede other species, even if these occur in larger numbers. The isolated strains, therefore, have more in

Table 3

Yeast strains from plate counts. Distribution to mull and mor soils

Group no.	Presumed identity	Number of strains from mull soils	mor soils	Total
2	*Pichia* sp. 1	—	1	1
4	*Cryptococcus laurentii*	—	2	2
5	,, *albidus*	—	2	2
7	*Torulopsis famata*	1	2	3
8	,, *aeria*	3	18	21
9	,, *ingeniosa*	—	1	1
10	,, *candida*	—	1	1
11	,, sp. 2	—	1	1
14	*Candida humicola*	11	1	12
15	,, *curvata*	1	1	2
16	,, sp. 1	36	45	81
17	,, sp. 2	2	—	2
18	,, sp. 3	9	10	19
19	*Trichosporon* sp. 1	6	13	19
20	*Rhodotorula mucilaginosa*	1	1	2
21	,, sp. 1	—	1	1
22	unknown	1	—	1
	total	71	100	171

Table 4

Yeast strains from enrichment cultures. Distribution to mull and mor soils

Group no.	Presumed identity	Number of strains from mull soils	mor soils	Total
1	*Saccharomyces fructuum*	—	1	1
3	*Lipomyces starkeyi*	2	—	2
4	*Cryptococcus laurentii*	2	1	3
5	*Cryptococcus albidus*	2	—	2
6	*Torulopsis* sp. 1	—	1	1
7	*Torulopsis famata*	—	1	1
12	*Candida solani*	—	1	1
13	*Candida krusei*	—	3	3
14	*Candida humicola*	7	8	15
20	*Rhodotorula mucilaginosa*	1	—	1
	total	14	16	30

common with the general concept of a "typical yeast", than have the plate count strains. 10 per cent of them were ascosporogenous, against 0.6 per cent of the strains from the plate counts, and 23 per cent were fermentative, against 2 per cent of the strains from the plate counts. On the other hand, the plate count strains had an appreciably stronger assimilative power than the enrichment strains. Most of them were able to assimilate all the carbon compounds tested.

As regards the physiological properties used for the standard descriptions, the yeasts predominant in soil constitute a homogenous group, in spite of large morphological differences. The typical soil yeasts are anascosporogenous and non-fermentative. They can assimilate a relatively large number of carbon compounds, and most of them can utilize nitrate as a source of nitrogen.

The role of yeasts in soil metabolism is not well known, and their importance, therefore, difficult to evaluate. However, so large a group of microorganisms cannot be quite insignificant. Many yeasts are able to decompose pectin, and many of them form lipase, as shown by the present investigations. Furthermore, they are fast-growing organisms, which rapidly decompose carbohydrates and other low-molecular carbon compounds produced during the degradation of cellulose and lignin, and they have other properties, e. g. the ability of growth over a very wide pH-range, which make them competitive in relation to other groups of soil microorganisms (cf. DI MENNA 1959).

Summary

The number of yeasts and yeast-like fungi in beech mull and beech mor soils was determined by plate counts on different media, although in an indirect way (see p. 44). The mull soils were found to contain from 0 to 100,000 yeasts per gm., and the mor soils from 0 to 500,000 per gm. Enrichment cultures with soil from the same localities showed the presence of yeasts in all samples examined.

A total of 171 yeast strains from the plate counts and 30 strains from the enrichment cultures were studied and described, and they were found to belong to 22 different species. 20 of these were demonstrated in the mor soils, against 13 in the mull soils. 9 species occurred only in mor soils, whereas only 2 species were found exclusively in the mull.

The predominant yeasts in these soils are anascosporogenous and non-fermentative, but with a rather strong assimilative power. Most of them belong to the genera *Torulopsis* and *Candida*.

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